

**Fig. S1**. Apparent FRET efficiencies  $Ef_D$  (blue) and  $Ef_A$  (red) as functions of the donor mole fraction  $x_D$  for homomers of 5-HT<sub>1A</sub> and 5-HT<sub>7</sub> receptors fitted for different n values by eq. 3.



**Fig. S2.** (A and C) Distribution of apparent FRET efficiencies  $Ef_D$  and  $Ef_A$  over  $x_D$  is shown for different receptor combinations. (**B** and **D**) Graphs show dependence of fitted K values (left axis) and errors of fit (black line, right axis) as function of FRET efficiency.



**Fig. S3.** Graph represents the simulation of the relative concentrations of hetero- and homodimers as well as corresponding monomers as function of the total concentration. The concentrations are scaled by the total concentration  $[1A]_{tot} + [7]_{tot}$ .



**Fig. S4.** (A) Co-injection of 5-HT<sub>7</sub> or 5-HT<sub>2C</sub> receptors does not influence distribution of GFP-tagged 5-HT<sub>1A</sub> receptor. (**B**) Bar graph illustrates quantification of relative fluorescences of 5-HT<sub>1A</sub>-GFP receptor injected either alone or together with 5-HT<sub>7</sub> or 5-HT<sub>2C</sub> receptors. (**C**) Two-electrode voltageclamp recordings from oocytes either injected with 5HT<sub>1A</sub> receptor alone (*left*), co-injected with 5-HT<sub>1A</sub> and 5-HT<sub>7</sub> receptors (*middle*) or with 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> receptors (*right*) reveal large inward currents upon hyper- and depolarizing steps from -140 to +60 mV (V<sub>H</sub>=-70mV) only in case of 5-HT<sub>1A</sub> receptor and 5-HT<sub>1A</sub>+5-HT<sub>2C</sub> receptors injections. Kir3.1/3.2 was present in all experiments. (**D**) Bar graph illustrates normalized current amplitudes from experiments with co-injection of 5-HT<sub>1A</sub> receptors with different GPCRs after activation with 5-HT. Reduction of current activation was only significant for co-expression of 5-HT<sub>1A</sub> with 5-HT<sub>7</sub> receptors. Each value represents the mean  $\pm$  S.E.M. (n = 4). A statistically significant difference between values is noted (\*\*, p < 0.01).



**Fig. S5.** (A) Analysis of siRNA directed against the 5-HT<sub>7</sub> receptor. The 5-HT<sub>7</sub>-YFP receptor was expressed in neuroblastoma N1E-115 cells, which then were co-transfected with scrambled or anti-5-HT<sub>7</sub> receptor shRNAs constructs. After SDS-PAGE gels were analysed by fluorescence scanner Typhoon 9400 (*upper panel*). Expression of GAPDH was analysed in parallel by western blot (*lower panel*). Representative image is shown. (**B-C**) Analysis of the expression level of 5-HT<sub>1A</sub> and 5-HT<sub>7</sub> receptors in hippocampus during development. Changes in the expression level of the 5-HT<sub>7</sub> (**B**) and 5-HT<sub>1A</sub> (**C**) receptors mRNA in the mouse hippocampus were determined at different stages of the postnatal development using real-time RT-PCR and  $\Delta\Delta C_t$  method. The 5-HT<sub>7</sub> receptor is strongly expressed at early postnatal stages and down-regulated during later stages of development, while expression of 5-HT<sub>1A</sub> receptor remains relative constant.